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Author(s)	UYEO, TOYOJI; HOSONO, SATORU; FERGUSON Jr, ALBERT B.
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Effect of Bleeding, Fracture and Diminished Oxygen Atmosphere on Lung Tissue Exposed to Fat

by

Toyoji Uyeo, Satoru Hosono, and Albert B. Ferguson, Jr.

Universty of Pittsburg School of Medicine Department of Orthopaedic Surgery

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Fat embolism as a pathologic and clinical entity has been recognized for many years. Its principal clinical manifestations are in the lung[10]. The symptoms appear after a latent period of twenty-four to forty-eight hours following injury. It is most common in severe injuries which involve closed fractures of the long bones.

A normal physiologic phenomenon is that of circulating fat in the blood stream. The development of the fat embolism syndrome may relate more to a depressed ability of the lung to cope with circulating fat than to an increased liberation of fat into the blood stream.

The accumulation of fatty acids and total lipids in the lung after fat injection is shown in these experiments. The experimental conditions have been varied by the addition of a hemorrhage, a fracture or a depressed PO_2 atmosphere for the animal.

With each of these additions the ability of the lung to metabolize and clear fat is shown to be depressed.

Fat Embolism Syndrome

Experimental evidence appears to show that the fat embolism syndrome is not due to solely to the injection of a bolus of neutral fat into the circulation[1]. Emson et al stated that fat embolism can be found in 80-100% of patients dying after a fracture[3),9)]. Despite the presence of fat in the tissues the incidence of fat embolism syndrome is relatively rare. If the present of fat alone were the cause of the syndrome a high incidence should be expected[4)].

In observing patients with the fat embolism syndrome, we have been impressed, not only with the tachycardia, tachypnea and petechiae, but also a serious hypovolemia. This is often unappreciated due to the closed nature of the injury. At autopsy, the lungs are destroyed and show intraalveolar hemorrhage with extensive tissue necrosis. These findings suggest that more than the mechanical obstruction of the lung capillaries by

Key Words Fat embolism Syndrome, Fatty acid toxicity, Lipase activity in the lung
Present address : Dr. Toyoji Uyeo, Department of Orthopaedie Sugery Kyoto University Medical School Sakyo-ku, Kyoto, Japan, 〒 606

fat embolus is involved. A chemical action of lipid break-down products appears to be involved in this destruction. Patients with the fat embolism syndrome exhibit a prolonged depression of the PO^2 of the blood[2]. This may sufficiently lower the physiologic ability of the lung to handle the excess fat to produce the syndrome.

PELTIER, et al reported that free fatty acids have a stronger taxic action than neutral fats on the lung epithelium[11]. Several reports have demonstrated that in the fat embolism syndrome, the serum lipase level is elevated in about half of the cases[12]. HALLGREN, et al reported that the fatty acid composition of the triglycerides in emboli were very similar to those present in bone marrow[7]

Putting the above observations together, the mechanism of the fat embolism syndrome appears as follows : induced fat released from the bone marrow into the circulation in severe injury accumulates in the lung where the fat is decomposed into fatty acids by lipase. Lung lipase activity is at least normal or higher than normal.

Conversely, the lung tissues are operating at less than normal efficiency if there is severe anemia or oxygen deficiency. As a result fatty acid is not metabolized at a normal rate in the lung and accumulates in it. This is intensified by blood congestion which occurs from mechanical obstruction of vessels by emboli. The local free fatty acid accumulation leads to tissue destruction by damaging the endothelium. The lung with its high lipase content normally clears fat and the breakdown products of fat without tissue damage.

Method

An investigation of the effect of severe soft tissue trauma and blood loss was made with particular reference to the ability of the lung to handle fat in the circulation under these conditions. The dose of injected fat was derived from the excellent article by ARMIN, et al[1]. He reported that 0.15ml. fat per kilogram body weight was enough to produce the pathological changes seen in the human fat embolism syndrome. Thirty adult rabbits were used and were divided into three groups for the experiment.

The first group of ten was subjected to bleeding of 18 ml. blood per kilogram body weight and injection of homogenous fat (0.15 ml/kg. body weight) through the vein of the ear. Second group of ten was subjected to fracture of the leg and the same volume of fat was injected. The third group of ten was kept in a chamber of low oxygen concentration for twenty-four hours to observe the influences of oxygen deficiency after the injection of fat.

Elevated free fatty acid levels in the lung of these rabbits were noted by comparing them to the levels in control rabbits.

Histological findings were assessed for lipase activity and tissue destruction in every lung. The total lipid content of the lung was determined along with the free fatty acid content.

Procedure

Adult albino rabbits weighing 1,840 grams to 5,560 grams were used in all experiments.

They were anesthetized lightly with sodium pentobarbital injected intravenously into an ear vein.

Preparation of the Fat

From a freshly killed rabbit, perirenal fat was quickly removed, minced and boiled in water. It was then squeezed through gauze and the clear supernatant fat was pipetted off and stored in a dessicator over sulphuric acid. The fat was prepared every ten days. The fatty acid concentration of this fat contained less than 1.0% as oleic acid.

"Bleeding" was carried out by withdrawing blood directly from the heart by anterior heart puncture.

An average of 18 ml/kg. of blood was aspirated in about three minutes and this volume was estimated to be 31% of the initial blood volume[1]. This was the maximum volume that could be aspirated consistent with animal survival. All rabbits showed severe tachycardia, tachypnea and anemic eyelids. They could not stand nor hold their heads up.

"Fracture" was performed on the right leg by manual force.

"Oxygen deficiency" was controlled in an atmospheric chamber by flowing mixed gasses of 80-100cc/min. O_2 and 800-1000ml/min. N_2. The animals exhibited labored respiration under these conditions. In an atmosphere of 60ml/min. O_2 and 1000ml/min. N_2 the rabbits could not survive.

Injection of the Fat

The fat was liquified at 40 °C and the required amount (0.15ml/per kilogram of body weight) drawn into a syringe. The fat was injected into the marginal vein and it was ascertained that the fat was carried by the blood stream down the vein in a succession of minute globules. The rabbits occasionally showed sneezing and increased respiratory rate after this injection.

Necropsy

After the indicated interval of time, animals were killed by the intravenous injection of a lethal dose of pentobarbital.

Immediately afterdeath, the trachea was tied to prevent collapse of the lung. The chest was opened and after tying the hilus of each lung, the lungs were separately excised. After weighing the lungs were fixed in formol calcium. One or two days later the fixed lungs were weighed again and 2 grams of fixed lung tissue was treated for fat extraction. This fixation procedure is important to avoid any loss of lipid and blood in the lung. It did not appear to alter the quantity of fat extracted[1].

Another block of lung tissue was used for frozen sections to evaluate lipase activities by "the tween method of PEARSE".

Fat Extraction

Total lipids were extracted from lung tissues by the modified FOLCH's method[6),8)].

Total lipids have been estimated by placing the solution obtained in a weighing bottle and allowing it to evaporate at 40°C with a vacuum. The residue was weighed.

Fatty acid Determination

The residue in a beaker from the total lipid determination was dissolved in 15ml. of 95% ethanol. It was heated and while hot, one drop of phenolphtalein was added and titration was done with 0.1 N NaOH. Titration values were compared with a blank. The calculations then became :

$$\frac{\text{ml. titration} \times \text{normality of alkali} \times 282 \times 100}{\text{Weight of lipid in mg}} =$$

% free fatty acid as oleic of total lipids.

The microequivalent of fatty acids per gram lung was also calculated.

Histological and Histochemical examinations

The lung tissue was fixed in formol calcium solution and frozen sections were made 15μ thick. The tween method of Pearse was used to stain for lipase activity. In order to compare the lipase activity as a function of incubation time the tissues were run at both 30 minutes and six hour intervals prior to staining.

Results

The free Fatty Acid Content of Lung

The free fatty acid content of the lungs (μ eq/gm. lung) after 6 hours, 24 hours and 48 hours showed elevated levels in both the bleeding and fracture group when compared

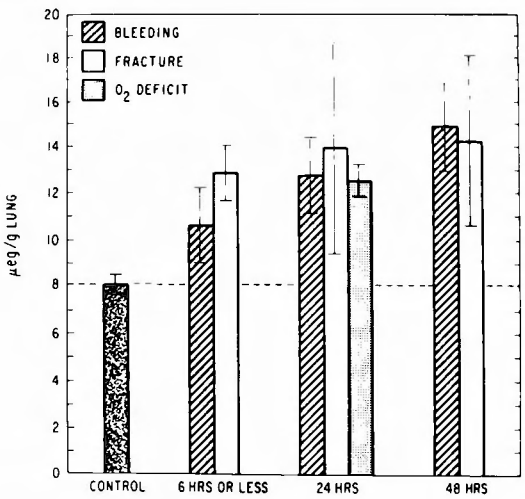

Fig 1. Absolute Volume Free Fatty Acid Related to Unit Gram of Lung Tissue
The absolute volume of free fatty acid related to lung tissues a measure of the interference with normal lung physiology caused by the variables introduced in this experiment. After fat injection oll groups show elevation of this important metabolic product-significant because it relates to tissue destruction of the lung parenchyma.The elevation continues at twenty-four hours and forty eight hours with diminished ability of the lung to clear free fatty acids. Bleeding, a fracture or a diminished PO_2 all cause his phenomenon.

to the control lung. When the animal was exposed to an oxygen deficient atmosphere a high free fatty acid content was also observed (Figue 1). There was about an 80% increase in free fatty acid content in the fracture and bleeding cases after 48 hours.

The percentage of free fatty acid to total lipid concentration in the lung did not show any consistent relationship (Fig. 2).

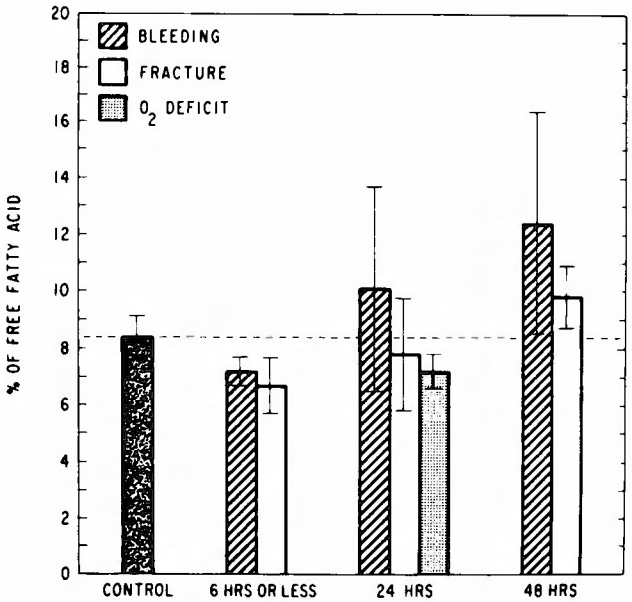

Fig. 2. Percent of Free Fatty Acid Related to Total Lung Lipid after Bleeding of Fracture or Lowered Oxygen Atmosphere.
The percent of free fatty acid in relation to total lung lipid is a measure of the lung's ability to metabolize and clear the fatty acids and thus the lung's physiologic efficiency in carrying out a normal process. After twenty-four and forty-eight hours the animals differing blood loss show a tendency to reduction of this ability and consequent free fatty acid accumulation.

Total Fat Content of Lung

Total fat content of the lung averaged 28mg/gms. in the controls. Total fat content values of 45mg/g lung in bleeding cases, 52 mg/g lung in fracture cases and 53mg/g lung in oxygen deficiency cases were obtained twenty four hours after fat injection. There was a decreased value at 48 hours after fat injection (Fig. 3).

Lipase Activity of the Lung

Lipase activity was not increased in the fat embolism lungs, but at least normal activity was maintained in all cases. In specimens showing areas of destruction this was noted where high lipase activity was obtained compared to surrounding normal portions of the lung (Fig. 4).

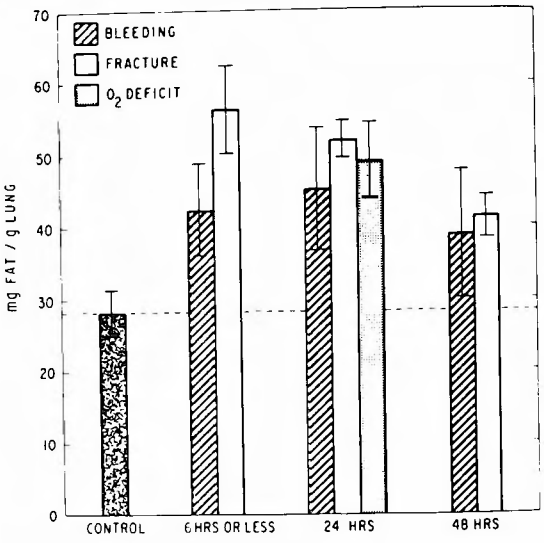

Fig 3. Fat Content Per Unit Gram of Lung Tlssue.
The fat content in a unit gram of lung tissue is increased after fat injection. This is increased in all animal groups at six hours, twenty-four hours, and forty-eight hours and reflects even in the later stage a decreased ability of the lung to metabolize and clear fat.

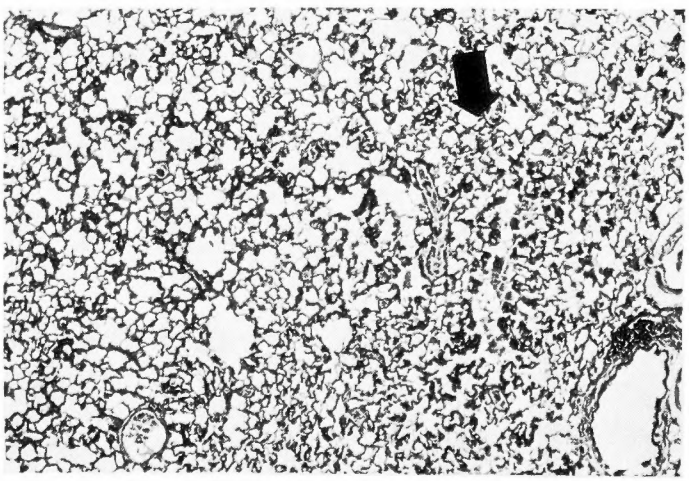

Fig 4. The destruction of lung tissue in the area which is indicated by the arrow is correlated with marked lipase activity which is recognized locally by darker brown stain than that of the surrounding area.

Discussion

A fat injection dose which would simulate fat embolism in man was used. ARMIN et al. demonstrated that this dose produced an embolism with histological changes in the lung which were compatible with that seen in man.

PELTIER, et al. have clearly demonstrated the toxic effect of free fatty acids on lung tissue with resultant edema and hemorrhage[11]. The toxicity is a function of the degree of unsaturation and may be a result of the affinity of fatty acids for calcium ions.

The lung tissue damage occurs in the presence of fatty acid accumulation in confirmed in these studies. PELTIER has also shown that the serum lipase is elevated in 50% of fat embolism cases[12] and Fonte has shown an elevation in fat injected rabbits[5]. In order to evaluate the fatty acid accumulation in the lung the absolute value in unit lung weight has been used here. This is more meaningful than relating the percentage of fatty acid to the total lipid. Tissue damage introduces a variable since it would be greatest in the areas of fatty acid concentration.

In these experiments the destruction of lung tissue was correlated with marked lipase activity. This suggests that there is an elevation of lipase activity in the lung with the appearance of circulating fat. The lipase may be secreted as an extra-respiratory function of the lung in response to the presence of the fat[12]. When the fatty acids liberated from the neutral fats by lipase accumulate they are toxic to the parenchymal cells leading to disruption of the alveolar-capillary membrane.

In the presence of an oxygen-deficient atmosphere, six animals died after twenty hours. Since this was not an immediate occurence it was presumed that the O_2 deficiency contri-

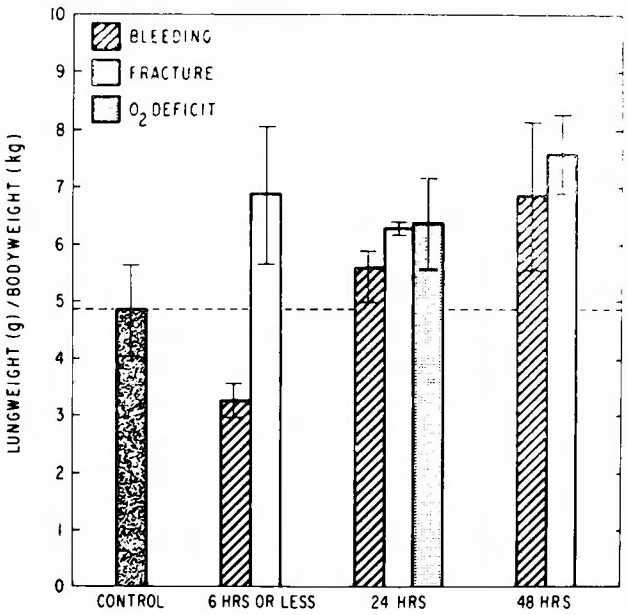

Fig 5. Lung Weight in Grams Related to Body Weight in Kirograms.
Lung weight in grams related to body weight in kilograms. The increase in lung weight may be ascribed to congestion in the lung. Its tendency to increase in both the twenty-four and and forty eight hour period after administration of fat intravenously. Animals who suffered blood loss, fracture or a dimished 0_2 atmosphere in addition alll showed this tendency.

buted to the demise. The O_2 deficiency could not be the sole cause, however, with this length of survival time. At autopsy pleural effusion and petechial hemorrhages on the pleural surface were seen at 24 and 48 hours.

These findings were not seen at 6 hours following injection. A gradual lung weight increase was observed after six hours except in the bleeding cases which showed a decreased lung weight possibly because of a total blood volume decrease reflected in the lung. (Fig. 5). The total lipid also increased from an average of 28.5mg. per gram lung to an abnormal level (Fig. 3). This finding was similar to the human fat embolism syndrome findings of ARMIN et al[1].

There are fundamentally two major theories accounting for the fat embolism syndrome: The mechanical theory is that fat emboli are due to a mechanical liberation of bone marrow fat[7]. The physio-chemical theory states that with trauma the body releases large amounts of catecholamines which markedly influence the lipid metabolism in the body. The embolic fat may be the end result of agglomeration of blood fat which is due to physio-chemical changes in the blood[9]. No matter how the fat globules appear in the lung, the injection of fat in this experimental model appears to duplicate the findings seen in clinical cases. It is obvious that the mere passage of fat through the lungs is not enough. Collins reported that the arterial oxygen tension of rabbits decreased 5 minutes after fat injection and recovered temporarily, then a prolonged low arterial oxygen tension was observed again. This biphasic pattern suggested an early mechanical effect and later a presumed chemical effect[2]. Excess blood loss and delayed replacement of blood volume to normal inhibits the ability of the lung to clear and restore the normal PO_2 situation[4]. A vicious cycle of lowered ability to clear fatty acids which was caused by oxygen deficiency and an oxygen deficiency which was caused by capillary obstruction initially and by tissue destruction later, may be a principal cause of the fat embolism syndrome.

It becomes very important if this is true to maintain normal lung physiology if the syndrome is to be prevented. Adequate replacement therapy must be used for the blood loss at the fracture site. In closed fractures this blood loss may be unappreciated. The PO_2 of the blood which is abnormally lowered at onset must be compatted early following injury[4].

Bibliography

1) Armin, J., Grant. R. T. Observations on gross pulmonary fat embolism in man and the rabbit.. Clin, Sci., **10** 441-464. 1951.
2) Collins, J. A., Caldwell, M. D. : Relationship of depot fat embolism to pulmonary structure and function in rabbits. Amer. J. Surg., **119** : 581-584, 1970.
3) Emson, H. E. : Fat emboism studied in one hundred patients dying after injury. J. Clin. Path. **11** : 28-35, 1958.
4) Ferguson, A. B., Jr. Fat embolism : A Preventable syndrome. South Aust. Clin., **6** : 2-3, 1972.
5) Fonte, D. A., Hansberger, F. X. . Pulmonary free fatty acids in experimental fat embolism. J. Trauma., **11** 668-672, 1971.
6) Folch, J., Lees, A. M., Meath, J. A. Preparation of lipid extracts from brain tissue. J. Biol. Chem., **191** 833-844, 1951.
7) Hallgren, B., Kerstell, J., Rudenstam, C. M. and Svanborg, A. : The influence of increased and

decreased plasma free fatty acids on the formation and composition of the fat emboli in the dog. Acta. Med. Scand. Suppl., **499** : 43-56, 1969.
8) Hayashi, K. : Preparation of lipid extracts from brain tissue. Brain and Nerve., **16** : 66-71, 1964.
9) Hernbon J. E. Riseborough, H. J. : Fat embolism, A review of current concepts. J. Trauma., **11** : 673-680, 1971.
10) Paredes, S., Comer F., Rubins S. ,Adler, F. . Fat embolism. Distribution of fat tagged with ^{131}I within the body of the rat at various time following intravenous injection. J. Bone Jt. Surg., **47A** : 1216-1220, 1965.
11) Peltier, L. F. Fat embolism. The toxic properties of neutral fat and free fatty acids. Surgery, **40** : 665-670, 1956.
12) Peltier, L. F., Scott, J. R. Fat embolism, Changes in the level of the blood lipase following the intravenous injection of neutral fat, fatty acids and other substances into dogs. Surgery, **42** : 541-547, 1957.
13) Peltier, L. F.: The diagnosis and treatment of fat emblism. J. Trauma., **11** : 661-667, 1971.

和文抄録

出血，骨折及び低酸素状態における脂肪塞栓の実験的研究

ピツッバーグ大学整形外科

上尾豊二，細野　惺，Albert B. Ferguson

脂肪塞栓に伴う症候群は外傷における重要な合併症であるが，実際上は比較的稀にしか臨床上見られない．しかし，Emson 等も述べるように骨折で死亡した患者の肺所見では80～100%に脂肪塞栓がみられる．それ故に脂肪塞栓症候が単に肺の脂肪の栓塞によってのみ生じるのなら，臨床的にこの症候群はもっと普通にみられてもよいはずである．脂肪塞栓で死亡した人の肺所見は，高度の肺組織の壊死を伴った著明な出血があり，単なる機械的な毛細管の閉塞よりも，脂肪の分解物の化学作用が組織破壊をもたらしたように見える．Peltier は free fatty acidsはneutral fats よりもはるかに肺上皮に対して毒性があることを報告した．又，脂肪塞栓においては，血中リパーゼの活性は約半数例において増加していると云われる．以上を総合すると，肺組織の破壊は肺に栓塞した脂肪がリパーゼで分解され，free の脂肪酸を生じ，これが貧血やショック状態においては消費され得ないため，肺局所に滞留し肺組織を障害すると推測される．これを確認するため家兎を用いて3群の実験を行った．第1群は体重kgあたり 20ml の脱血を行い，ショックを生じた家兎に体重kgあたり0.15mlの脂肪を注入した．第2群は下腿骨々折を生じた後に同様に脂肪を注入した．第3群は脂肪注入後，気密室で低酸素状態に維持した．そしてこれから6時間，24時間，48時間後の肺の組織所見とリパーゼとの関係，単位肺重量あたりの脂肪及び脂肪酸の含量の測定を行った．その結果いずれの群においても free の脂肪酸の増量が証明され，組織所見では肺内の局所的なリパーゼの活性増加と，同部の組織破壊が伴っている所見がみられた．